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Characteristics of Young Infants in Whom Human Parechovirus, Enterovirus or Neither Were Detected in Cerebrospinal Fluid during Sepsis Evaluations

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Abstract

Background—Human Parechovirus (HPeV) causes central nervous system (CNS) infection in infants. To further understand HPeV CNS infection, we describe its clinical, laboratory and epidemiologic characteristics from a Midwestern U.S. tertiary care center. Because HPeV CNS infections have appeared clinically and seasonally similar to enterovirus (EV) infections, we retrospectively compared characteristics of young infants undergoing sepsis evaluations in whom HPeV, EV or neither were detected in CSF.

Methods—HPeV real-time RT-PCR assay was performed on frozen nucleic acid extracts of CSF specimens submitted for EV RT-PCR assay from children seen at our hospital in 2009. HPeV genotyping was performed by sequencing of the viral protein 1 (VP1) region. Clinical data were abstracted from medical records retrospectively for EV-positive, HPeV-positive and age-matched controls in whom neither virus was detected from CSF testing.

Results—HPeV was detected in 66/388 (17%) CSF specimens while EV was detected in 54/388 (14%) from June through October 2009. Genotyping identified HPeV3 in 51/66 (77%) positive CSF specimens. Males predominated (61%) with the most common presenting symptoms (91%) being fever and irritability. All HPeV positive patients were <5 months of age. Eight required admission to the pediatric intensive care unit. In multivariate analysis, lower peripheral WBC counts with lower ALC values, higher maximum temperatures, longer fever duration, absence of pleocytosis, and longer hospitalization were independently associated with HPeV patients compared to patients with EV or patients negative for both HPeV and EV.

Conclusions—Our data indicate that HPeV3, an emerging CNS pathogen of infants in the United States, should be considered in sepsis-like presentation even without CSF pleocytosis.

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Addition of HPeV RT-PCR to EV RT-PCR assay for CSF specimens of patients less than 6 months of age could reduce hospital stay and costs while improving clinical management.

Keywords

Human Parechovirus; Enterovirus; Sepsis-like illness; Children; Cerebrospinal fluid

INTRODUCTION

The human parechoviruses (HPeV) are a recently recognized genus belonging to the family *Picornaviridae*. They were originally classified within the *Enterovirus* (EV) genus with HPeV types 1 and 2 known as echovirus 22 and echovirus 23, respectively. The reclassification arose from sequence analysis in the early 1990s demonstrating distinct genetic differences between echovirus 22 and EV genus members.¹ To date, 16 HPeV types have been characterized (www.picornaviridae.com).²

The clinical and epidemiologic patterns for EV have been well studied, as they are common human pathogens. In the neonatal/infant population specifically, EV presentations are commonly that of a nonspecific febrile illness for which a sepsis evaluation is often performed in infants less than three months of age.³ Less common but more severe presentations include frank encephalitis, hepatitis, sepsis syndrome and/or myocarditis.⁴

Clinical presentations of HPeV mostly appear similar to EV. HPeV1 has been the most commonly reported type. Both HPeV1 and HPeV2 have been associated with mild gastrointestinal and respiratory symptoms.^{5,6} HPeV3, however, has been associated with more severe clinical manifestation in the form of sepsis-like and central nervous system (CNS) illnesses particularly in neonates and infants less than 3 months of age.⁷⁻⁹ HPeV3 was initially isolated from a patient in Japan during 1999 and has been detected with increasing frequency over the last decade in Asia, Europe, Canada, South America and more recently in the United States.⁷⁻¹⁶ However, much remains to be discovered regarding the characteristics of HPeV3 infection as well as those of HPeV types 4-16.

We previously reported clusters of HPeV3 CNS infection in a Midwestern United States pediatric population during 2006-08.⁷ Renaud *et al* demonstrated a peak in HPeV CNS disease in Seattle, Washington, in 2009.¹⁶

In the current study we describe the clinical, laboratory and epidemiologic characteristics of predominantly HPeV3 CNS infection during 2009. Additionally, we present comparisons between patients with HPeV3 CNS infection to those with EV and those with similar presentations but without detectable HPeV or EV (controls). Our aim was to identify characteristics that could assist clinicians in differentiating between HPeV, EV and those with neither virus in the CSF.

MATERIALS AND METHODS

Clinical Specimens

We included 388 CSF specimens from children in this study (range = 1 day to 18 years; median age = 45 days; average age = 769 days). These CSF specimens were previously submitted for EV real-time RT-PCR assay during June through October 2009 as part of standard of care for patients treated at Children's Mercy Hospitals and Clinics (CMH).

Laboratory Testing for HPeV

All previously tested CSF specimens were assayed for HPeV. Total nucleic acids (TNA) were extracted from CSF by using the EasyMag automated extractor (bioMerieux, Durham, NC) and aliquots were stored frozen at -80°C since 2009. The extracts were initially tested at CMH by a two-step real-time RT-PCR as described by Benschop et al. (2008), with modifications as previously reported.^{7,17} Total nucleic acid extracts from HPeV-positive CSF specimens were later forwarded to the Centers for Disease Control and Prevention (CDC) and tested by parechovirus one-step real-time RT-PCR¹⁸ to confirm HPeV positivity. The VP1 region was then sequenced by a nested PCR assay (Nix, et al., 2010) to determine HPeV type. All sequences were deposited in the GenBank database, accession numbers JF919622-JF919672.

Phylogenetic Analysis

Complete HPeV3 VP1 sequences (678 nucleotides) from the CMH strains, other VP1 clinical sequences from the CDC sequence database, and strains from GenBank were aligned using Clustal W.²⁰ Phylogenetic relationships were reconstructed by the neighbor-joining method²¹, using evolutionary distances computed by the Kimura-2 parameter method.²³ Pairwise, percent nucleotide identities were calculated from the Clustal W alignment using MegAlign (DNASar, v.8.1.2; Madison, WI).²²

Identification of Study Groups

Despite testing CSF for HPeV from all patients less than 19 years of age, HPeV was detected only in patients < 6 months of age, therefore the comparator groups included all EV patients < 6 months of age and one each age-matched patient with neither EV nor HPeV detected (controls). Patients who had CSF enterovirus PCR testing performed were thus grouped into three groups: 1) HPeV-positive group (n=66), 2) EV-positive group (n= 47), and 3) control group (n=66).

Clinical Characteristics of Infants Examined

We documented age and month of diagnosis, gender, clinical symptoms, laboratory results, chest radiograph results, use and duration of antimicrobials and length of hospitalization. Fever was defined as parent-reported (historical fever) or documented temperature (in emergency department or hospital records) of $>38^{\circ}\text{C}$ for patients <30 days of age and $>38.3^{\circ}\text{C}$ for those >30 days of age.⁷ Hypothermia was defined as a parent-reported or documented temperature $<36.7^{\circ}\text{C}$.⁷ Length of hospitalization was assigned by the number of

calendar days the patient spent in the hospital. Clinical symptoms were obtained either from caregiver report or hospital documentation by providers.

Meningitis was defined by age-specific white blood cell (WBC) count parameters. CSF pleocytosis was considered present with >28 WBCs in the first 30 days of life or >8 WBCs after 30 days of life.⁷ CSF glucose was considered abnormal if it was below threshold detection by assay (<20 mg/dL) or <50% of concurrent serum glucose. CSF protein was considered elevated at >150 mg/dL in neonates <10 days and at >58 mg/dL in patients older than 10 days.⁷ Normal peripheral WBC count was defined as 5,000 to 15,000/ μ L. Neutropenia was defined as an absolute neutrophil count (ANC) <500 WBC/ μ L and lymphopenia as lymphocyte counts <1,500/uL. Normal platelet count was considered between 100,000 and 450,000 cells/ μ L with thrombocytopenia and thrombocytosis defined as less and greater than these values, respectively. The study was approved by the Institutional Review Board at CMH.

Statistical Analysis

GraphPad InStat version 3.06 was used for statistical analysis. Mean and standard deviations were calculated for continuous variables. An unpaired t-test was used for comparing two-group continuous variables. This form of comparison was also utilized when comparing the three groups individually with one another. One-way Analysis of Variance (ANOVA) was used when collectively comparing the three groups of continuous variables. The Tukey-Kramer Multiple Comparison was subsequently utilized for post-test analysis. A two-sided Fisher exact test was used to compare categorical variables. SigmaStat (Systat Software, Inc., Chicago, IL) was used for multivariate stepwise analysis. A *p* value < 0.05 was considered significant.

RESULTS

HPeV Prevalence and Genotype Analysis

HPeV was detected in 66/388 (17%) CSF specimens tested by the two-step HPeV real-time RT-PCR while EV was detected in 54/388 (13%) from June through October 2009. The majority of HPeV-infected children were male (40/66) with 82% presenting at less than 60 days of age and 100% less than 6 months of age. HPeV was detected from June to October with 52% of cases occurring during the month of August (Figure 1).

Total nucleic acids (TNA) from the 66 HPeV-positive CSF specimens were later sent to the CDC and retested by a one-step real-time RT-PCR, which detected parechovirus in 56/66 (85%) specimens. Parechovirus genotyping was not attempted on the ten TNA extracts that tested negative with the CDC one-step RT-PCR test. Specimens positive by two-step RT-PCR assay had lower Ct values (median 33.6; range 22.2 to 40) versus specimens positive by two-step PCR only and negative by one step RT-PCR (median 38.6; range 35.6 to 40). Fifty-one specimens (91%; 51/56) positive by one-step RT-PCR assay were successfully typed by sequencing the VP1 region and all of these were identified as HPeV3. Three of the CDC real-time RT-PCR positive specimens lacked sufficient volume to attempt genotyping. Two specimens failed sequencing reaction and had high real-time C_T values (39.3 and 38.3),

indicating low viral genome copy number. Forty-five of 51 HPeV3 VP1 gene sequences were full length. The six that were less than full length were not included in the phylogenetic analyses. Overall, the HPeV3 VP1 sequences shared 93% nucleotide identity (NT ID). All of the HPeV3 viruses in our study were closely related to other viruses detected recently in the United States, with the exception of one virus that was clearly of Asian origin (Figure 2). CMH 2009 HPeV3 viruses and the US HPeV3 viruses from 2005 shared 95.6-100% NT ID. With the exception of the CMH HPeV3 virus of Asian origin, the CMH HPeV3 viruses were 98.2-100% identical to one another. The strain of Asian origin (11620) shared 99% NT ID with two 2005 HPeV3 viruses from Thailand and only 94-95.6% NT ID to the other CMH HPeV3 viruses. The father of this patient had recently traveled in Asia.

Characteristics of HPeV-positive, EV-positive and control (Neg-HPeV/EV) patients

There was no difference in gender predominance when the HPeV group was compared to EV and Neg-HPeV/EV groups (Table 1). However, the age of presentation for the EV group was younger than that of the HPeV group ($P = 0.048$). The frequency of the presenting symptoms of irritability and fever was not different between HPeV and the other groups. However, fever was of longer duration and the maximum in-hospital temperature was also higher in HPeV patients compared to both EV and Neg-HPeV/EV groups ($P < 0.0001$). On day of discharge more HPeV patients (12/63; 19%) remained febrile than did the EV patients (5/47, 11%). One patient each in the HPeV and EV groups experienced seizure activity.

All EV-positive and Neg-HPeV/EV patients were hospitalized while 63/66 HPeV-positive patients were hospitalized. While eight HPeV-positive patients (12%) required care in the Pediatric Intensive Care Unit (PICU), only one EV-positive patient and no Neg-HPeV/EV patients required PICU care. Six HPeV-positive patients admitted to the PICU exhibited clinical signs of septic shock with decreased perfusion and hypotension requiring volume resuscitation. Of the remaining two HPeV PICU patients, one was admitted for severe neutropenia and the other for a generalized seizure.

CSF pleocytosis was rare (2%) in HPeV patients compared to EV (41%) and Neg-HPeV/EV patients (13%) ($P < 0.001$) (Table 1). Additionally, the average CSF WBC count of HPeV patients was lower than that of EV patients ($P < 0.001$). There was no difference in CSF WBC counts between HPeV and Neg-HPeV/EV patients ($P = 0.075$). The CSF glucose was lower in EV compared to HPeV patients ($P < 0.001$) while the protein in EV was higher ($P < 0.001$). This significantly higher protein difference persisted even when accounting for age-specific values of normal. HSV PCR testing on CSF was ordered in 16 EV-positive patients, 18 HPeV-positive patients and 15 control patients. All were negative for HSV.

CBC results between the three groups were also different (Table 1). The average peripheral WBC count for HPeV patients was lower than that of both EV and Neg-HPeV/EV patients ($P < 0.001$). The ANC and ALC (absolute lymphocyte count) were correspondingly lower in HPeV than both other groups (Table 1). The lower mean AMC (absolute monocyte count) of the HPeV group was not statistically significant ($P = 0.40$). There was no significant difference in CRP values among those tested.

There was no difference between antibiotic use or length of antimicrobial therapy between HPeV and the other two groups. Average duration of hospitalization was longer in HPeV patients compared to the EV and Neg-HPeV/EV groups, ($P = 0.005$).

DISCUSSION

This study adds to mounting evidence that HPeV-CNS infection is clinically relevant to neonatal and infant populations. We report an HPeV infection rate of 17% (66/388) in CSF originally submitted for EV testing from June through October 2009 in the Midwest United States. HPeV prevalence was as high as or higher than EV has been for most recent years. All HPeV were detected in summer through early autumn.⁷ All typeable HPeV strains from CSF (n=51) were HPeV3.

The world-wide distribution of HPeV3 is illustrated by the phylogenetic tree, using CMH and other selected complete VP1 gene sequences (Figure 2). All but one 2009 CMH isolate clustered with recent US HPeV3 viruses (98.2-100 percent identical), while European and Asian viruses formed separate clusters. We speculate that the CMH HPeV3 strain that was similar to Asian isolates was transmitted from father to child, given the father's recent Asian travel and the virus' genetic relationship (99% NT ID) to Thailand and Japan strains.

Our seasonality data confirms previous observations from across the globe including Europe^{8,11} and recently also from Seattle, WA during May 2009 to May 2010.¹⁶ Despite indications of summer through fall seasonality, year-round testing is needed to ensure that HPeV does not also occur outside this interval.

We observed little activity in 2006 (2% prevalence; 4/218 CSF) and 2008 (0% prevalence; 0/242) in contrast to 2007⁷ and 2009. Similar intermittent HPeV activity was noted, but in even-numbered years, in the United Kingdom and the Netherlands.^{8,9} Thus, HPeV3 seems to cause periodic CNS infection outbreaks. Interestingly, our 17% CNS HPeV infection rates are identical in 2007⁷ and 2009.

Males predominated among the combined 124 HPeV patients from 2007 and 2009, but males also predominated in our EV control group. Male gender does not differentiate HPeV from EV. The 2009 EV group (31.3 days) are at least 10 days younger when compared to our 2009 (40.8) and 2007 (46.2 days) HPeV groups. The clinical importance of this difference is unclear.

CSF from all children less than 19 years of age was tested, but most HPeV-positive patients were <60 days of age, and all <5 months old. The most frequent presenting symptoms were fever and irritability in the current and prior HPeV patient groups. This may not be surprising given that these symptoms without a known focus of infection generally provoke a sepsis workup, including CSF testing.

Abnormal CSF was rare in both our 2007 and 2009 HPeV groups, with pleocytosis in <10%. Low peripheral WBC count, ALC and ANC were noted in both years of HPeV groups. The normal CSF findings and low peripheral WBC and ALC distinguish the HPeV groups from

the EV group. Our HPeV patient findings are similar to a Seattle, WA report.¹⁶ Our data do not provide a reason for this more intense fever with leukopenia.

HPeV patients had longer duration of fever with a higher maximum temperature during hospitalization. In both the EV and HPeV groups, there were likely some post-discharge febrile days which we did not capture. However, proportionally more HPeV patients were still febrile on discharge than EV patients. Thus we likely would have missed more febrile days in the HPeV group than the EV group, resulting in underestimation of the duration of fever in HPeV-infected patients and supporting the idea that HPeV caused a longer duration of fever than EV.

Non-U.S. studies show that HPeV CNS infection can be similar to EV infections^{7,8,11}. But in one Netherlands study,¹¹ 8/11 neonates with HPeV infection from 1994 to 2006 showed mild-to-severe CNS white matter abnormalities in MRI studies. Three of eight had neurodevelopmental delay at follow-up. In our study only 6/66 of our HPeV patients had neuro-imaging studies and all were normal. Normal neurologic examinations and/ no CSF pleocytosis likely made CMH providers feel that further CNS imaging was unwarranted. We did not perform developmental follow-up. This may be of low yield given none of our patients had abnormal discharge neurologic examinations.

The one extra mean hospital day compared to the EV group may have been due to longer and higher maximum temperatures in HPeV patients. Clinicians likely were uncomfortable discharging highly febrile young infants, who unbeknown to them had HPeV infection (because results were unavailable during the hospitalization). In contrast, a laboratory-confirmed diagnosis of EV and the shorter fever duration in EV patients likely gave clinicians' confidence to discharge EV patients sooner.¹⁹ Alternatively, HPeV patients may have been more ill-appearing than EV patients regardless of fever.

Like the EV group and when compared to the HPeV group, the Neg HPeV/EV group had significantly shorter duration of fever and hospitalization, lower maximum temperature in the hospital, and higher peripheral leukocyte values, including total WBC counts, ANC's and ALC's. The shorter hospital stay may again be due to the clinician's greater level of confidence in discharging infants who by 48 hours of hospitalization were no longer febrile and who had negative sepsis workups.

Our data suggest that a positive HPeV RT-PCR in CSF is sufficient to explain the symptoms of fever and irritability in infants <6 months of age. We detected no dual CNS infections (HPeV plus other viral or bacterial agents). No HPeV patient had a bacterial bloodstream infection. However, two HPeV-positive children had concomitant urinary tract infections (one *S. aureus* and one *E. coli* infection), but these were evident within 24 hours of admission.

Limitations to our study include its retrospective nature. Chart documentation may not be as complete as data from prospective studies. Additionally, testing CSF originally sent for testing in EV season potentially precludes us from identifying HPeV with other clinical presentations or outside the typical EV season. In addition, since EV types were not

identified in this study, variable clinical presentations could be expected in other years due to variability in EV types circulating from year-to-year.

In conclusion, HPeV CNS infection can be the sole cause of sepsis-like syndrome in young infants. It should be considered particularly when initial laboratory data show a normal CSF without pleocytosis but low peripheral WBC, ALC and/or ANC counts. Hospitalized HPeV patients appear to have an extra febrile day compared to EV patients or those with no proven CSF viral pathogen. Multiple days with in-hospital temperatures greater than 39°C were characteristic of HPeV but not EV infections.

Based on this and our prior 2007 study, we suggest potential benefits to routine HPeV RT-PCR on CSF of <6 month old infants undergoing a sepsis workup during summer/fall in the United States. Clinicians could decide on earlier discharge for patients with confirmed HPeV. CMH now offers routine HPeV RT-PCR. We plan to prospectively measure the impact of this HPeV testing on patient management when there are sufficient numbers of HPeV cases for analysis.

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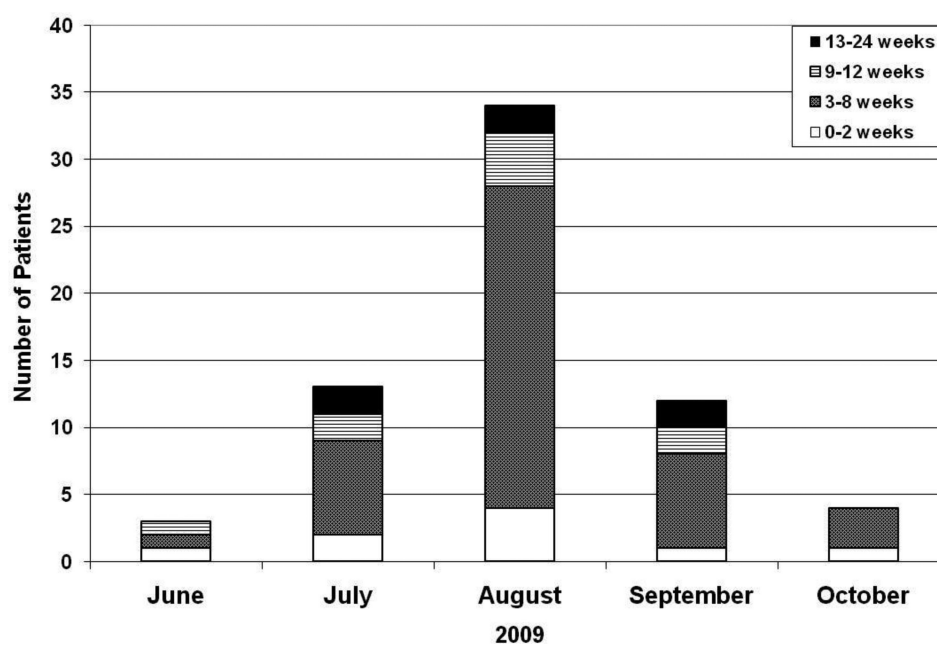


Fig 1.
HPeV-CNS infections in children from Kansas City, 2009.

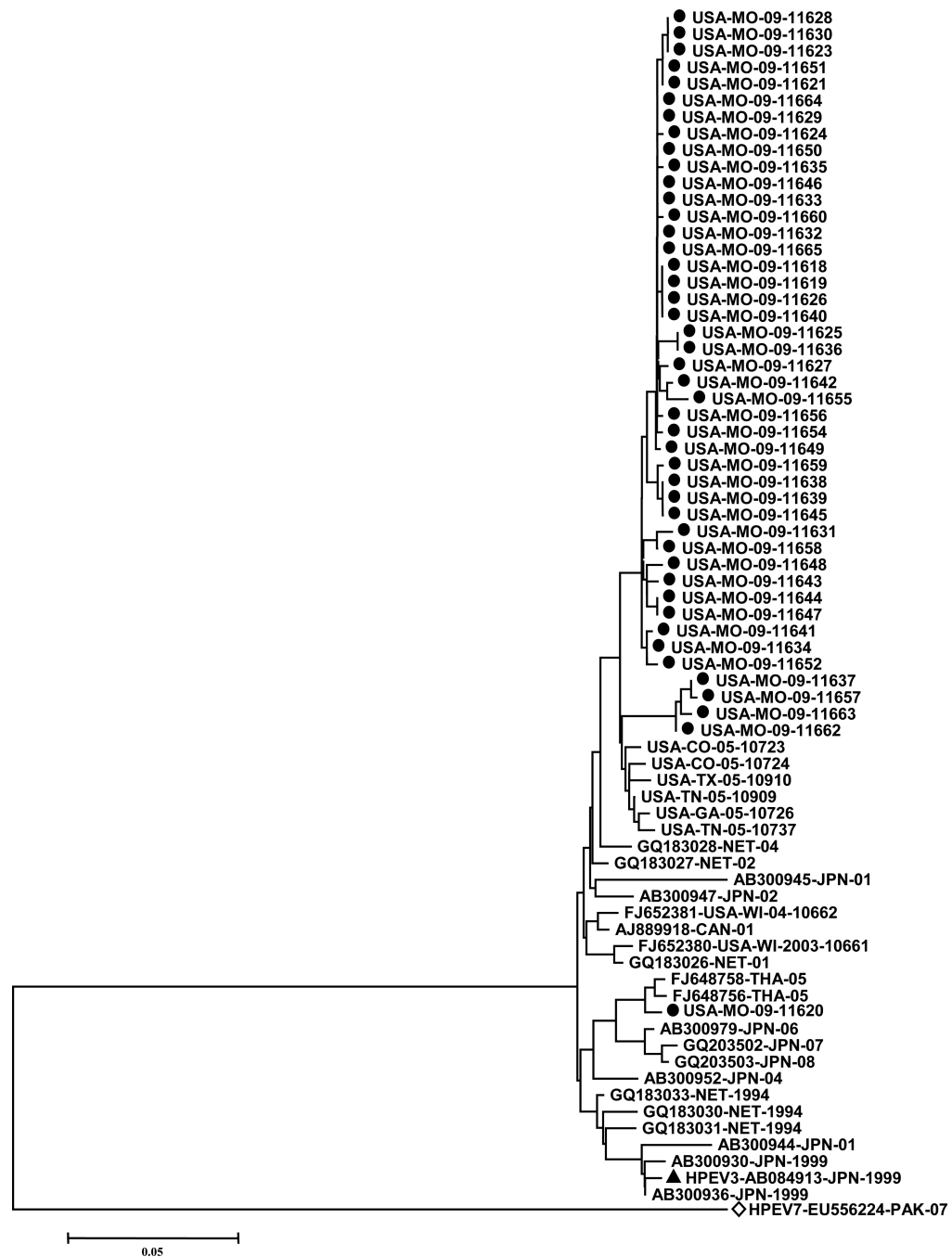


Figure 2.

HPeV3 complete VP1 sequences (678 nucleotides) are shown on a neighbor-joining tree. Kansas City viruses are highlighted with filled circles, the prototype HPeV3 virus with a filled triangle, and the outgroup (HPeV7) with a hollow diamond. Country abbreviations are: USA, United States; NET, Netherlands; JPN, Japan; CAN, Canada; THA, Thailand; PAK, Pakistan.

Table. 1

Significant Clinical and Laboratory Findings in Patients with HPeV, EV or neither (Neg) detected in CSF from Infants < 6 months of Age.

| | HPeV | EV | Neg | <i>P</i> Value ^{\$} |
|---------------------------|----------------|---------------|---------------|------------------------------|
| Number of Patients | 66 | 47 | 66 | NA |
| Age (in days) | 40.8 ± 26.6 | 31.3 ± 18.6 | 42.8 ± 27.8 | 0.0481 |
| Male/Female | 40/26 | 27/20 | 45/21 | 0.467 |
| Inpatient Charges | 14,177 ± 9,231 | 9,547 ± 5,075 | 9,829 ± 5,673 | 0.0004 |
| Hospital Days | 3.9 ± 1.4 | 3.2 ± 0.8 | 3.1 ± 0.8 | 0.0054 |
| PICU Days | 0.23 ± 0.72 | 0.06 ± 0.44 | 0 | 0.0237 |
| Tmax Hospital * | 39.2 ± 0.7 | 38.4 ± 0.9 | 38.0 ± 0.9 | <0.0001 |
| Days of Fever * | 2.7 ± 1.1 | 2.0 ± 0.9 | 1.6 ± 1.9 | <0.0001 |
| CSF WBC | 3.9 ± 11.6 | 144.0 ± 338.5 | 7.8 ± 13.1 | <0.0001 |
| CSF Pleocytosis * | 1 | 18 | 8 | <0.001 |
| CSF Glucose | 49 ± 6.3 | 42.2 ± 5.8 | 46.3 ± 7.7 | <0.0001 |
| CSF Protein | 41.5 ± 21.8 | 59 ± 28.8 | 44.7 ± 23.2 | 0.0007 |
| Peripheral WBC * | 5.81 ± 2.25 | 9.21 ± 3.32 | 10.14 ± 5.57 | <0.0001 |
| ANC | 2.90 ± 1.78 | 4.36 ± 2.56 | 3.97 ± 3.52 | <0.0136 |
| ALC * | 1.79 ± 1.04 | 3.80 ± 2.29 | 4.39 ± 2.12 | <0.0001 |

^{\$} significant difference in univariate analysis

* significant difference in multivariate analysis